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SHORT COMMUNICATION

CA9 and *CHRNB1* were correlated with perineural invasion in Taiwanese colorectal cancer patients

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Abstract Colorectal cancer (CRC) is a significant public health problem worldwide. The recent identification of genes overexpressed in CRC, combined with advances in molecular biology techniques, provides opportunities to establish more sensitive, specific, and cost-effective ways of identifying local recurrence or metastasis. This study explored the overexpression of prognosis-associated mRNAs in human CRC by using a well-established enzymatic chip array platform. An analysis of 15 CRC cancer tissue specimens and their normal adjacent tissues revealed that 10 genes—*CA9*, *CD55*, *CHRNB1*, *CK20*, *ELAVL4*, *hTERT*, *KCTD2*, *MUC1*, *PSG2*, *TMPO*—were upregulated in CRC cancer tissue by microarray and bioinformatics analysis. A gene chip including 10 candidate genes was constructed to investigate the circulating tumor cells in blood specimens of 156 CRC patients and further validated by reverse transcription-polymerase chain reaction. The correlations between clinicopathologic features and the gene expression of 10 candidate genes are compared using the Chi-square test. Results from this study demonstrated that the overexpression of *CA9* and *CHRNB1* genes

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correlated with perineural invasion (both $p < 0.05$). The *CHRNB1* overexpression may affect the opening of an ion-conducting channel across the plasma membrane of tumor cells. The *CHRNB1* gene may be a suitable new marker to predict disease prognosis for Taiwanese patients with CRC.

Introduction

Up to 25—40% of colorectal cancer (CRC) patients who undergo curative resection subsequently develop metastatic disease. Undetected micrometastasis may play a key role in the metastasis of CRC patients. One of the major causes of failure is the presence of disseminated tumor cells shed from the primary carcinoma into the circulation prior to, during, or after surgery. Circulating tumor cells (CTCs) were first discovered in the blood of a cancer patient (*postmortem*) by Ashworth.[1] The detection of CTCs in the peripheral blood of CRC patients was reported to have important prognostic and therapeutic implications. Our recent investigations have demonstrated that the persistent presence of postoperative CTCs is a poor prognostic factor for patients with CRC after curative resection by membrane array-based multimarker assay.[2–4] The aim of this study is to explore the overexpression of prognosis-related mRNAs by detecting the CTCs in human CRC.

Materials and methods

An analysis of 15 CRC cancer tissue specimens compared with their normal adjacent tissues revealed that 10 genes were upregulated (gene expression ratio of cancer tissue to paired normal tissue >2) by microarray and bioinformatics analysis. A weighted enzymatic chip array including 10 prognosis-related genes was used to investigate CTCs. Consecutive 156 patients were prospectively enrolled in surgical units in Taiwan. These patients were required to have cytologically/histologically confirmed CRC. The associations of each gene expression with the clinicopathological data of patients were further analyzed.

Results

Eighty-eight men (56.4%) and 68 women (43.6%) were recorded. Thirty patients were subsequently diagnosed with stage I—II, 121 with stage III, one with stage IV, and four with unknown stage of the disease. Overall, 90 of 156 (57.7%) patients were identified to have a tumor size smaller than 5 cm; 66 of 156 patients (42.3%) were found to have a larger tumor size (≥5 cm). There were statistical correlations between perineural invasion and *CA9* (carbonic anhydrase 9) gene overexpression ($p = 0.044$) and *CHRNB1* [cholinergic receptor, nicotinic, beta 1 (muscle)] gene overexpression ($p = 0.047$).

Discussion

CA9 expression has been identified in numerous cancer types (head and neck squamous cell carcinoma,[5] cervical cancer[6]) and is generally associated with hypoxia and poor prognosis in nonsmall cell lung cancer.[7]

Nicotinic acetylcholine receptors encoded by *CHRNB1* are ligand-gated ion channels that modulate key physiological processes ranging from neurotransmission to cancer signaling. These receptors are activated by the neurotransmitter, acetylcholine, and the tobacco alkaloid, nicotine. Recently, the gene cluster encoding the alpha3, alpha5, and beta4 nicotinic acetylcholine receptor subunits attracted heightened interest after a succession of linkage analyses and association studies identified multiple single-nucleotide polymorphisms in these genes that are associated with an increased risk for nicotine dependence and lung cancer.[8,9] Polymorphisms of nicotinic acetylcholine receptor subunits alpha or beta were potential risk factors of cervical carcinogenesis.[10] Perineural invasion was grossly underreported in CRC and could serve as an independent prognostic factor of outcomes in CRC patients, and perineural invasion should be considered when stratifying CRC patients for adjuvant treatment.[11] The results of the present study suggest that *CA9* and *CHRNB1* genes were related to perineural invasion with statistical significance. They can be potential prognostic markers for Taiwanese patients with CRC.

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